Targeting Solute Carrier Transporters through Functional Mapping

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Solute carrier (SLC) transporters are emerging drug targets. Identifying the molecular determinants responsible for their specific and selective transport activities and describing key interactions with their ligands are crucial steps towards the design of potential new drugs. A general functional mapping across more than 400 human SLC transporters would pave the way to the rational and systematic design of molecules modulating cellular transport.

Challenging Drug Targets

SLC transporters mediate the transport of a broad range of solutes, such as ions, nutrients, and metabolites across biological membranes. In human, dysregulation of the homeostasis of the transported substrates, has been associated with multiple diseases and disorders, such as cancers. Additionally, SLCs play an essential role in the absorption, distribution, metabolism, and elimination, of therapeutic drugs. Thus, SLCs are key drug targets [1,2], that remained understudied until recently [3]. Understanding these complex biological systems requires the description of many aspects of their functioning (e.g., interactions with ligands and with protein partners, conformational changes and kinetics of transport, response to cofactors, and differential expression in different cell types). These different aspects can be probed by various technologies, including structural determination, genetic editing, metabolomics, various animal models, chemical biology, basic biochemistry, etc. Among them, structure-based techniques are commonly used to improve our understanding of substrate specificity determinants. As such, they bear a great potential to guide the rational design of prospective new drugs. Importantly, these methods are most powerful when integrating experimental information together with data generated *in silico* [4,5]. The experimental determination of SLC 3D structures is challenging due to the inherent difficulty of expressing and purifying membrane proteins in their native state. As a result, about 100 structures of human SLCs have been resolved to this day, and they represent only 25 unique proteins. Fortunately, several structures of prokaryotic homologs are available, and represent good templates to build homology models of SLCs [6]. These structures are postulated to describe several intermediate states of the transport cycle, thus improving our understanding of the transport process, and providing opportunities to design conformation specific modulators.

The transport mechanism is by essence a dynamic process. According to the alternating access paradigm, a transporter needs to go from an outward, to an inward conformation, passing through intermediate states such as the occluded state, where the substrate is isolated within the binding site. This requires local gating movements as well as global movements of two domains with respect to each other. Three distinct SLCs folds have been predominately described, and a transport mechanism has been associated to each of them (Figure 1A) [7]: (i) The rocker-switch corresponds to the fold transporting, for example, human di/tri-peptide [peptide transporter 1 (PepT1), SLC15A1], or glucose [glucose transporter 1 (GLUT1), SLC2A1]. (ii) The gated-pore or rocking bundle is adopted for instance, by the neutral amino acid 1 transporter [(LAT1), SLC7A5], or the serotonin transporter [(SERT), SLC6A4]. (iii) The elevator mechanism is operated among others by the glutamate transporter [excitatory amino acid transporter 1 (EAAT1)]. SLCs are comprised of transmembrane α-helices (usually 10–14), regrouped into pseudo-repeats related by a symmetry axis (Figure 1B).

Plenty of efforts have been made to describe key structural determinants, defining the transport specificity of important therapeutic targets among SLCs. For example, the structural basis for the distinct transport activities the creatine transporter [(CreaT), SLC6A8], exerts on its substrates using molecular modeling methods [8], has recently been described. CreaT belongs to the SLC6 family and operates transport via the gated-pore mechanism. It is a pharmacologically relevant target, as it is responsible for the intracellular uptake of creatine, a crucial metabolite regulating ATP homeostasis in tissues with high-energy demand. Briefly, it has been shown that the most potent CreaT inhibitors exhibit a 4.5 Å long carbon linker between the guanidine and carboxylate moieties, to maintain optimal interactions with a deprotonated cysteine (C144) unique to this transporter, and a glycine (G71), conserved in the γ-aminobutyric acid (GABA) transporter subgroup of the SLC6 family (Figure 1C). Such specific features of the binding site complemented by the specific ligand's scaffolds, confer to CreaT very unique transport properties not found in the other members of the SLC6 family.

Despite similar findings reported on other SLCs, we are still far from a complete understanding of how SLCs select and transport their substrates. In particular, about one third of SLCs called 'orphans' do not have any known ligands nor function [9].

A Long and Complex Evolutionary History

The 456 known human SLC transporters are grouped in 65 families based on their sequence and functional similarities. A characteristic property of the SLC 'superfamily' is its very high sequence variability. Many families share very weak sequence

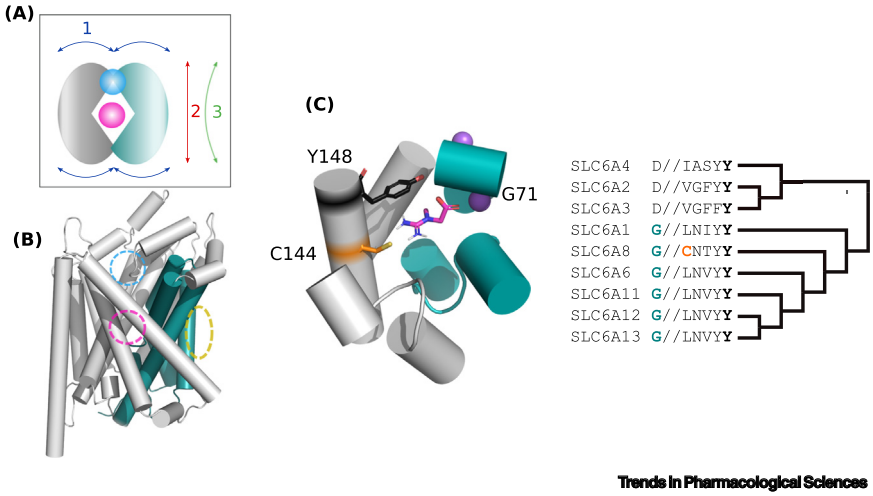

Figure 1. Structure- and Sequence-Based Characterization of Solute Carrier (SLC) Transporters. (A) Schematic representation of the three main SLC transport mechanisms, with the two domains of the transporter colored in gray and cyan. In the rocker-switch (1), both domains oscillate back and forth whereas in the elevator (2), and gated-pore (3), one domain opens and closes the binding site, while the other domain remains static. The pink sphere indicates the transported substrate, while the cyan sphere highlights the binding site of the allosteric modulators documented for the elevator and gated-pore mechanisms. (B) Cartoon representation of the 3D homology model of the creatine transporter (CreaT, SLC6A8), as an illustrative example of the so called 'LeuT-fold', operating via the gated-pore mechanism [8]. The static scaffold and mobile domains are colored in gray and cyan, as in panel (A). The pink and blue broken-lined circles indicate the orthosteric and allosteric sites, as in panel (A), while the yellow circle indicates a binding site for cholesterol. (C) Close-up view of the CreaT binding site, with the docked creatine shown as pink unbroken lines. Key residues are labelled: Y148 acts as a gate, while C144, and G71 have been identified as being involved in the selectivity of CreaT towards creatine. The evolutionary conservation of the three positions is illustrated with the multiple sequence alignment on the right. The phylogenetic tree linking the sequences was adapted from [20].

similarities, sometimes even if they adopt the same fold and transport mechanism [10]. While the evolutionary origin of several main SLC families can be dated prior to the divergence of bilaterian species, a large number of SLC genes seem to have evolved very rapidly and to be species- or lineage-specific [11]. Moreover, the existence of three distinct folds and their involvement in the transport of the same substrates, suggest independent evolutionary scenarios, leading to different structural and dynamical solutions for performing the same function. Interestingly, while some unrelated SLC proteins may transport the same substrates, others belonging to the same family may transport different substrates. This raises the issue of the detection of signals relevant to substrate specificity from sequence data. Evolutionary conservation is a widely recognized proxy for function. Residues under strong selective pressure are expected to play essential roles for the folding of the protein and its transport activity. More variable residues may also play crucial roles in ensuring and maintaining protein function, by optimizing the transport of a certain substrate over others (Figure 1C). To identify such residues, it is necessary to account for interdependencies between residues (e.g., compensatory mutations) and to group sequences according to their evolutionary history [12]. This was recently illustrated on the G protein-coupled receptor (GPCR) superfamily, where selectivity signatures could be identified between evolutionary related receptors coupling the same G protein [13]. In the case of SLC transporters, deciphering the complexity and multiplicity of the evolutionary events leading to present-day proteins is challenging. This difficulty calls for the development of computational approaches exploiting both sequence- and structure-based information. Specifically, the high structural similarities observed between many, sometimes highly divergent SLCs, could be advantageously used to establish a holistic mapping of sequence positions between them (Figure 2).

Functional Mapping

Despite striking commonalities observed between SLCs so far, very few functional and structural connections have been established across different families. Of particular interest for medicinal chemistry are allosteric mechanisms, by which the binding of a molecule at a site distant from the ligand (orthosteric) binding site, modulates the function of the transporter. Such mechanisms have been described for the elevator and gated-pore transport processes [14–17] and have been suggested for the rocker switch [18]. More specifically, some allosteric inhibitors have been identified for the glutamate transporter EAAT1 (SLC1A3) [14] and the SERT (SLC6A4) [15,17]. Interestingly, in both cases, the allosteric inhibitors bind at the interface between the scaffold and mobile domains, hence preventing the conformational change. Additionally, an allosteric activator has been revealed for the glutamate transporter EAAT2 (SLC1A2) [16]. It was suggested to 'unlock' several intermediate states, leading to an increased rate of conformational change. In other biological systems such as GPCRs, designing allosteric modulators has proven to be an efficient strategy, to target protein subtypes more selectively than with orthosteric ligands. These results show the clinical potential of developing such modulators for transporters. New allosteric ligands for transporters could then be used: (i) as chemical tools to improve our understanding of allostery as an emerging concept for transporters; and (ii) as new potential hits for optimization.

More generally, it is hypothesized that it is possible to systematically transfer functional

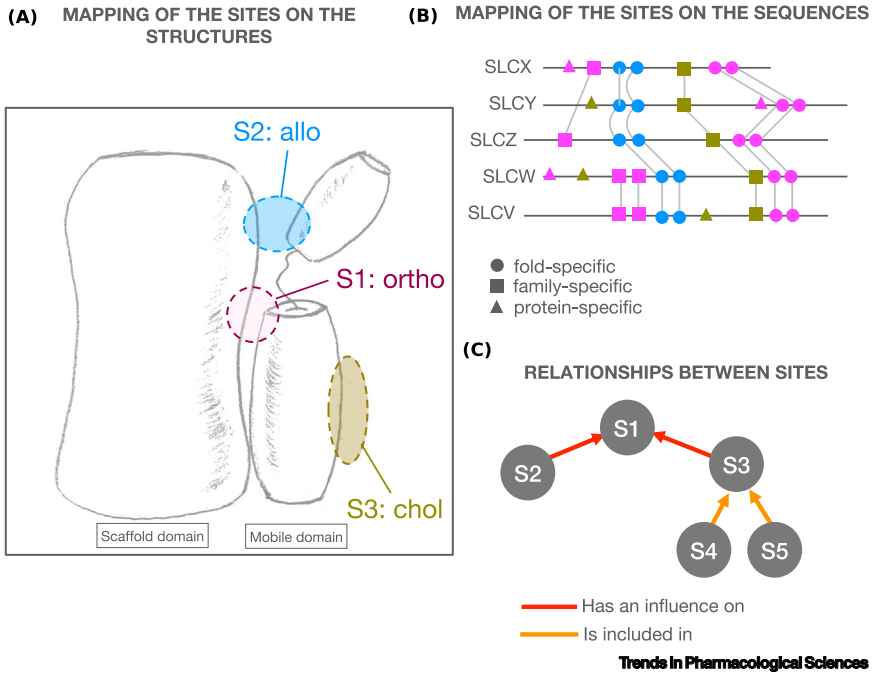

Figure 2. Functional Mapping of Solute Carrier (SLC) Transporters. The LeuT-fold is shown as an example to illustrate the kind of functional map one could build. (A) Schematic representation of the fold with several sites mapped from different SLC families (same sites as in Figure 1). (B) Several SLC sequences are shown, with the positions involved in the sites S1, S2, and S3 highlighted in color. The links between the positions, are derived from structural similarities between the transporters. The shapes of the symbols indicate the level of conservation of the amino acids. (C) Network depicting the relationships between the different sites. Other sites and other types of relationships may easily be included.

and structural annotations between SLCs [19]. Such holistic mapping could provide new concepts to tackle drug discovery on these key targets. Several layers, with a gradual increase in precision, can be envisioned. The first one would reveal regulatory mechanisms shared by all transporters (e.g., allosteric regulation through the scaffold and transport domains interface). The second one would be fold specific, and requires a more detailed description of the structural basis of transport modulations as reported in the literature (e.g., polarity, protonation, and shape of the binding site, orthosteric or allosteric). The third one would uncover specificity determinants, responsible for different SLC members of the same family transporting very diverse substrates, and reveal family-specific 'signatures', at the residue resolution (Figure 2). Notably, the second and third layers can be thought of as complementary, and integrate structure and sequence-based approaches.

Concluding Remarks

Collecting the state-of-the-art information on the structure–function relationships of SLCs in an integrative manner, is a key step towards a global understanding of their function. It shall also help to design more systematic and efficient strategies, to specifically target each transporter, and to control the activity of these key pharmacological proteins with new drugs.

Here we propose several actions to be taken towards achieving this goal. First, it is believed that learning from best-studied systems can provide insights into how to efficiently tackle drug discovery for SLC transporters. A few examples of how research on GPCRs can guide and inspire research on SLCs has been provided here. Second, it is strongly believed that connecting transporters sharing similar folds (despite belonging to distinct families) is essential. This has been illustrated in this article by pointing out fold-dependent commonalities in transport modulation.

Third, it is suggested that combining structure-based methods with evolutionary analysis, can considerably improve the identification of key determinants, at the residue level, of SLCs functions. Similarly to the commonalities described for orthosteric sites of LeuT-fold transporters [19], it is expected that such mapping will reveal throughout the entire structure, conserved residues involved in function, as well as nonconserved residues involved in specificity, including secondary sites. This information would permit the targeting of these sites in a more specific and efficient manner.

Finally, integrating the proposed actions constitute a novel and unique method, which would permit the building of a holistic functional map of SLCs.

Ultimately, this comprehensive approach could be applied beyond characterizing allosteric regulation, and would help to better understand how this complex family of proteins functions.

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